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Research article

Foliar application of effective microorganisms on pea as an alternative fertilizer

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Abstract – Effective microorganisms are cultures of coexisting beneficial microorganisms predominantly consisting of species of photosynthetic and lactic acid bacteria, yeast and actinomycetes, that could be used for alternative crop production. I studied the effect of soil and foliar application of these microorganisms on crop growth, yield and nodulation in pea (*Pisum sativum* L.), in soils amended with *Trifolium alexandrinum* L. green manure, farmyard manure and NPK fertilizers. The results show that foliar application of effective microorganisms enhanced nodulation, using NPK amendment, reaching a 217% increase for nodule number and a 167% increase for nodule biomass. Similarly, a grain yield increase of 126% for NPK amendment and of 145% for green manure amendment was found after foliar application of effective microorganisms. These findings show that foliar application of effective microorganisms in combination with proper soil amendment can improve nodulation and yield in pea.

effective microorganisms / soil application / foliar spray / soil amendments / pea / nodulation / yield

1. INTRODUCTION

Fertilizers as a source of plant nutrients and pesticides as plant protection measures are being used to increase crop production. However, imbalance and frequent use of these agrochemicals have polluted the environment to a great extent. Concern is growing that food produced under such farm management may not be safe or of good quality. This has shifted the scientific approach towards some alternative measures (Ferron and Deguine, 2005; Shaxson, 2006). In the recent past some successful efforts have been made to at least partially substitute chemicals with natural substances to minimize the bad effects of the former. One such effort was made by Higa (1989), who isolated some beneficial microorganisms from the soil and called them Effective Microorganisms. Effective microorganism culture consists of co-existing beneficial microorganisms, the main ones being the species of photosynthetic bacteria, viz. *Rhodopseudomonas plastris* and *Rhodobacter sphacrodes*; lactobacilli, viz. *Lactobacillus plantarum*, *L. casei* and *Streptococcus lactis*; yeasts (*Saccharomyces* spp.), and *Actinomycetes* (*Streptomyces* spp.), which improve crop growth and yield by increasing photosynthesis, producing bioactive substances such as hormones and enzymes, controlling soil diseases and accelerating decomposition of lignin materials in the soil (Higa, 2000; Hussain et al., 2002). In Pakistan this technology of nature farming was introduced in 1990 by the Nature Farming Research Center, University of Agriculture, Faisalabad. Numerous field and greenhouse trials are indicative of the benefits of this technology for crop production, as a probiotic in poultry and livestock rations, and to enhance the composting and recycling of municipal/industrial wastes and effluents (Hussain et al., 1995, 1999). At present, the Nature Farming Research and Development Foundation is disseminating this technology throughout the country. Three commercial products, viz. EM Bioaab, EM Biovet and EM Biocontrol, have been introduced to farmers by this organization. EM Bioaab is used in agricultural crops along with organic manures as a substitute for chemical fertilizers. EM Biovet is used in livestock and poultry production, while EM Biocontrol is used in crops, vegetables and orchards for prevention and remedy of diseases and insect pest attack (Hussain et al., 2002).

When effective microorganism cultures are applied to soil they stimulate the decomposition of organic wastes and residues, thereby releasing inorganic nutrients for plant uptake. The majority of the scientists who are engaged in promoting this technology have no doubt that plant growth is just as good or better and the quality of plant products is superior to conventional farming (Hussain et al., 1999; Yamada and Xu, 2000; Xu et al., 2000; Iwaishi, 2000). However, the experiences of some authors revealed that the effect of effective microorganisms on crop yield was usually not evident or even negative, particularly in the first test crop. It is often difficult to establish the predominance of effective microorganism cultures in soil with only a single application and during only one season. Certain soil properties and the indigenous soil microbial populations are often constraints to the establishment of these

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microorganisms (Bajwa et al., 1995; Javaid et al., 1997). Studies have shown that these constraints can be overcome through periodic repeated applications of effective microorganisms, at least during the first few years (Javaid et al., 2000a, b, c, 2002; Javaid and Bajwa, 2002). Foliar application of effective microorganisms avoids many of the biotic and abiotic factors and constraints of the soil environment. Widdiana and Higa (1998) studied the effect of foliar application of effective microorganisms on the production of garlic, onion, tomato and watermelon, compared with the recommended application of chemical fertilizers. Generally, vegetable yields were higher with foliar-applied effective microorganisms compared with the chemical fertilizer. Similarly, Xiaohou et al. (2001) reported that sprinkling with effective microorganisms could increase yield and quality of various crops, fruits and vegetables. The purpose of this study was to investigate the comparative effects of foliar and soil applications of effective microorganisms on growth, yield and nodulation in pea, in soils amended with different organic and inorganic fertilizers.

2. MATERIALS AND METHODS

2.1. Soil and environmental characteristics

The soil used in the experiment was loamy textured with 0.9% organic matter, pH 8.2, 0.05% nitrogen, 14 mg kg^{-1} available phosphorus and 210 mg kg^{-1} available potassium. The experiment was conducted during November–January 2004–2005. The rainfall during November, December and January was 2.5, 12.5 and 20 mm, the mean minimum temperature was 57, 49 and 48 °F and the mean maximum temperature was 77, 67 and 65 F, respectively. The day duration during November, December and January ranged from 10 h 15 min to 10 h 55, 10 h 06 min to 10 h 16 min, and 10 h 06 min to 10 h 39 min, respectively. The weather was mostly clear with bright sunshine.

2.2. Soil amendments

Earthen pots of 20 cm diameter and 30 cm deep were filled with 2.5 kg soil each. The soil was amended either with farmyard manure @ 7 g/100 g, *Trifolium alexandrinum* green manure @ 7 g/100 g or NPK fertilizers. A basal dose of 40 mg kg^{-1} N as urea, 30 mg kg^{-1} P_2O_5 as triple superphosphate and 20 mg kg^{-1} K_2O as potassium sulfate was supplied to the pot soil of NPK treatments. NPK fertilizers were mixed three days prior to sowing. All the pots were irrigated with tap water and left for one month for decomposition of organic materials.

2.3. Treatments and experimental design

There were four treatments for each of the three soil amendment systems. These were (i) control, (ii) foliar spray with effective microorganisms, (iii) soil irrigation with effective microorganisms, and (iv) foliar spray plus soil irrigation with effective microorganisms. Each treatment was replicated thrice. Pots were arranged in a completely randomized design on a bench in a wire-netting house under natural environmental conditions.

2.4. Effective microorganism application

Effective microorganism culture under the commercial name of EM Bioaab was obtained from the Nature Farming Research and Development Foundation, Faisalabad, Pakistan. The stock culture was diluted to prepare 0.2% solution by adding tap water. The fresh solution was used immediately after preparation. The respective pots of the treatments with soil effective microorganism application in all the three soil amendment systems were irrigated with a 0.2% dilute solution 15 days prior to sowing. Each pot received 1 L of dilute solution. These pots were further supplemented with 1 L 0.2% effective microorganism solution fortnightly throughout the experimental period. Plants of the treatments with foliar application of effective microorganisms were sprayed fortnightly with 0.2% solution just to moisten the plant surface, throughout the experimental period. (Chowdhary et al., 2002; Yadav, 2002).

2.5. Sowing of seeds and harvesting schedule

Seeds of pea, surface-sterilized with 1% sodium hypochlorite solution, were sown in all the pots. Initially four seeds were sown in each pot, which were thinned to two uniform seedlings after one week of germination. Plants were harvested 35 and 70 days after sowing, corresponding to the flowering and fruit maturity stages, respectively. At each harvest date plants were carefully uprooted and thoroughly washed under tap water. Nodules were separated from roots and counted. Shoot length of each plant was measured. Root, shoot, pods and nodules were dried at 60 ºC until constant weight. At the maturity stage data regarding number of pods per plant, pod length, number of seeds per pod and grain yield per plant was also recorded.

2.6. Statistical analysis

There were three replicates per treatment and two plants per replicate pot. For each treatment mean data per plant were calculated for various studied parameters. Data regarding various root and shoot growth, nodulation and yield parameters were subjected to analysis of variance followed by Duncan's Multiple Range Test to delineate mean differences (Steel and Torrie, 1980).

3. RESULTS AND DISCUSSION

3.1. Effect of soil and foliar application of effective microorganisms on shoot and root growth of pea

The effect of soil amendments on shoot length was significant (Tab. I). Maximum shoot length was recorded in NPK fertilizer amendment at both the harvest stages. The effect of effective microorganism application on shoot length was also significant (Tab. I). In NPK amendment, at the flowering stage, the highest shoot length was recorded in soil application of microorganisms that was significantly greater than foliar spray and soil plus foliar treatments. However, at maturity the highest shoot length was observed in the foliar spray treatment, which was significantly greater than the rest of the treatments. In farmyard manure the lowest shoot length was observed in soil plus

Table I. Analysis of variance for various growth and nodulation parameters of pea as affected by soil amendments and effective microorganism application at two growth stages.

Trait	df	Mean squares				
		Shoot length	Shoot dry wt.	Root dry wt.	No. of nodules	Nodule dry wt.
Treatments	23	30**	0.36**	0.005**	149**	1460**
Harvest (G)	1	28**	0.008ns	0.022**	946**	11628**
Amendments (A)	2	28**	0.50**	0.007**	228**	3027**
Effective microorganisms (E)	3	101**	1.44**	0.015**	170**	945**
G×A	2	21**	0.065*	0.001**	131**	2266**
G×E	3	22**	0.50**	0.004**	82**	1042**
A×E	6	18**	0.16**	0.002**	97**	630**
G×A×E	6	14**	0.06**	0.002**	72**	271**
Error	48	1.25	0.009	0.0002	12	42
Total	72					

*, **: Significant at $P \leq 0.01$ and 0.001, respectively.
ns: Not significant.

Table II. Effect of soil and foliar application of effective microorganisms on shoot and root growth of pea after 35 and 70 days of sowing, in soils amended with NPK fertilizers, farmyard manure and green manure.

Treatments	35 days after sowing			70 days after sowing		
	Shoot length (cm)	Shoot dry wt. (g/plant)	Root dry wt. (g/plant)	Shoot length (cm)	Shoot dry wt. (g/plant)	Root dry wt. (g/plant)
NPK fertilizers						
Control	36 ab	1.21 a	0.09 ab	35 bc	1.04 c	0.13 b
Foliar spray	32 bc	1.04 ab	0.07 cd	39 a	1.77 a	0.21 a
Soil application	38 a	0.93 b	0.07 cd	36 b	0.75 e	0.09 c
Foliar + Soil	29 d	0.54 cd	0.05 ef	27 g	0.56 f	0.05 d
Farmyard manure						
Control	35 bc	1.20 a	0.1 a	34 cd	0.98 cd	0.09 c
Foliar spray	34 bc	0.70 c	0.05 ef	32 e	0.87 de	0.08 c
Soil application	34 bc	0.67 c	0.04 fg	33 de	0.41 gh	0.08 c
Foliar + Soil	30 d	0.56 cd	0.03 gh	28 g	0.48 fg	0.04 d
Green manure						
Control	35 bc	1.11 ab	0.09 ab	28 g	1.09 bc	0.12 b
Foliar spray	33 bc	0.50 cd	0.06 de	33 de	1.16 b	0.12 b
Soil application	31 cd	0.43 d	0.02 h	30 f	0.47 fg	0.05 d
Foliar + Soil	31 cd	0.70 c	0.05 ef	30 f	0.32 h	0.05 d

In a column values with different letters show a significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

foliar treatment at both the harvest stages. In green manure amendment, there was not any significant difference in shoot length among the treatments at the flowering stage. However, at maturity the foliar spray treatment exhibited the highest and significantly greater shoot length than all other treatments (Tab. II).

The effect of soil amendments as well as effective microorganism application was significant (Tab. I). Shoot dry biomass in all the effective microorganism treatments, either foliar spray or soil application, was markedly lower than corresponding control treatments at the flowering stage in all the three soil amendment systems. The effect was more pronounced in farmyard and green manures as compared with NKP fertilizers (Tab. II). Similarly, at maturity soil and foliar plus soil applications invariably and significantly depressed the studied parameters in all the soil amendments (Tab. II). These results are in agreement with the findings of earlier authors, who found that the effect of soil application of effective microorganisms on crop growth and yield was usually not evident or even negative particularly in the first test crop (Bajwa et al., 1999;

Table III. Effect of soil and foliar application of effective microorganisms on nodulation of pea after 35 and 70 days of sowing, in soils amended with NPK fertilizers, farmyard manure and green manure.

Treatments	35 days after sowing		70 days after sowing	
	No. of nodules/plant	Nodule dry wt. (mg/plant)	No. of nodules/plant	Nodule dry wt. (mg/plant)
NPK Fertilizers				
Control	25 ab	90 a	6 d	15 de
Foliar spray	17 c–e	60 b	19 ab	40 a
Soil application	19 b–e	30 d	8 d	14 e
Foliar + Soil	13 e	50 bc	6 d	11 e
Farmyard manure				
Control	32 a	80 a	21 a	30 b
Foliar spray	21 b–d	40 cd	7 d	15 de
Soil application	25 ab	60 b	13 c	17 c–e
Foliar + Soil	23 bc	50 bc	16 bd	25 bc
Green manure				
Control	18 b–e	30 d	19 ab	15 de
Foliar spray	15 de	10 e	23 a	23 b–d
Soil application	16 c–e	30 d	6 d	18 c–e
Foliar + Soil	14 de	15 e	7 d	17 c–e

In each column values with different letters show a significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

Table IV. Analysis of variance for various yield parameters of pea as affected by soil amendments and effective microorganism application.

Trait	df	Mean squares			
		No. of pods	Pod length	No. of seeds/pod	Grain yield
Treatments	11	4.2**	0.98**	2.70**	0.15^{**}
Amendments (A)	2	0.17^{ns}	0.33^{ns}	1.41^{ns}	4.02^{ns}
Effective microorganisms (E)	3	10.6**	2.4**	8.50^{**}	0.46^{**}
A×E	6	2.2**	0.47*	0.22^{ns}	4.63^{**}
Error	24	0.27	0.11	0.63	7.73
Total	36				

*, **: Significant at $P \leq 0.01$ and 0.001, respectively.
ns: Not significant.

Xu, 2000), possibly because introduced effective microorganisms have to face competition from soil indigenous microflora (Bajwa et al., 1995). Generally, crop growth and yield with effective microorganism application tends to increase gradually as subsequent crops are grown (Javaid et al., 2000a, b, c). According to Kinjo et al. (2000), the lack of consistency in results of the experiments regarding effective microorganism application may be due to variable cultural conditions employed in previous studies. Imai and Higa (1994) stated that the observed decline in crop yields can often be attributed to the fact that soils where conventional farming is practiced have become disease-inducing or putrefactive soils from long-term use of pesticides and chemical fertilizers. Consequently, it takes time to establish a disease-suppressive or zymogenic soil. Until this conversion process is completed, it is virtually impossible to exceed crop yields that were obtained with conventional farming methods. In the present study, foliar spray with effective microorganisms significantly enhanced shoot biomass over control in NPK fertilizer amendment, while in other soil amendments the effect was insignificant at the maturity stage (Tab. II). Earlier, Widdiana and Higa (1998) in Indonesia reported that generally growth of vegetables such as garlic, onion, tomato and watermelon was higher with foliar-applied effective microorganisms compared with chemical fertilizer.

Soil amendments and effective microorganisms showed a significant effect on root biomass (Tab. I). At the flowering stage all the effective microorganism treatments showed significantly depressed root biomass as compared with control. (Tab. II). Soil plus foliar application of microorganisms also exhibited a similar effect on root biomass in all the three soil amendments at the maturity stage. Foliar application, however, resulted in a significantly enhanced root dry biomass in NPK at this later growth stage (Tab. II).

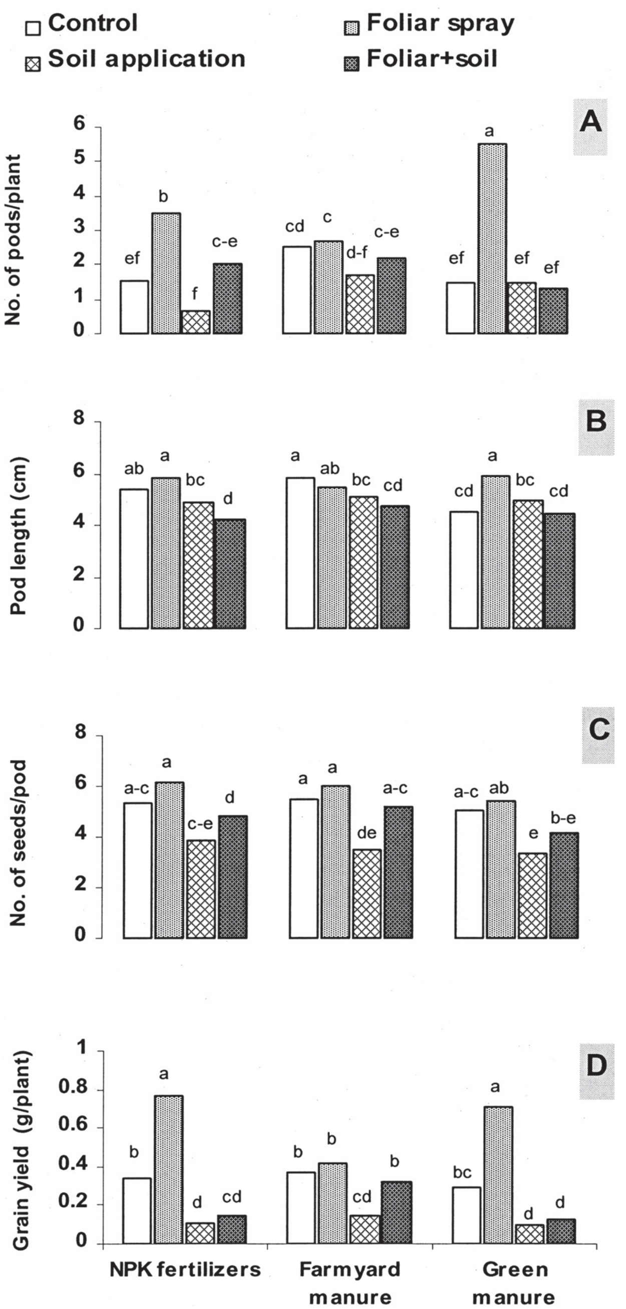

Figure 1. Effect of foliar spray and soil application of effective microorganisms on different yield parameters of pea in soils amended with NPK fertilizers, farmyard manure and green manure. Values with different letters show significant difference ($P = 0.05$) as determined by Duncan's Multiple Range Test.

3.2. Effect of soil and foliar application of effective microorganisms on nodulation of pea

The effect of growth stage, soil amendments and effective microorganisms, as well as their various interactions, was significant both on number and dry biomass of nodules (Tab. I). At the flowering stage effective microorganism application depressed the nodulation and the maximum number of nodules was recorded in uninoculated control in all the three soil amendments. The difference between control and microbial inoculated treatments was more pronounced in NPK and farmyard manure amendments than green manure amendment (Tab. III). At maturity, foliar spray of effective microorganisms significantly enhanced the number of nodules in NPK fertilizer amendment. A similar but insignificant increase in the number of nodules was also recorded due to foliar spray in green manure amendment. Soil and soil plus foliar application of effective microorganisms depressed nodulation in all the three soil amendment systems at this growth stage (Tab. III). The effect of foliar spray and soil application of effective microorganisms on nodule biomass in different soil amendment systems was generally similar to that of nodule number (Tab. III).

3.3. Effect of soil and foliar application of effective microorganisms on yield of pea

The effect of effective microorganism application on various yield parameters, viz. number of pods per plant, pod length, number of seeds per pod and grain yield per plant was significant, while the effect of soil amendments on these parameters was insignificant (Tab. IV). Soil application of microorganisms significantly reduced the number of pods per plants in NPK and farmyard manure amendments. Conversely, a significant increase in the studied parameter was recorded due to foliar spray in NPK and green manure amendments. Soil plus foliar application of microorganisms showed an insignificant effect on pod number in all the three soil amendment systems (Fig. 1A).

Pod length in NPK and farmyard manure amendments was significantly depressed in soil plus foliar treatment. Foliar application in green manure amendment resulted in an increase in the studied parameter (Fig. 1B).

An insignificant increase in number of seeds per pod was observed due to foliar spray of effective microorganisms in all the three soil amendments. By contrast, soil application invariably and significantly suppressed the studied parameter in all amendments. Soil plus foliar application of effective microorganisms failed to alter significantly the number of seeds per pod achieved in the corresponding uninoculated control treatments in any of the three soil amendment systems (Fig. 1C).

In NPK and green manure amendments, treatments with foliar spray of effective microorganisms exhibited maximum and significantly greater grain yield as compared with all other treatments. Both soil and soil plus foliar application of effective microorganisms depressed the grain yield (Fig. 1D). It seems probable that foliar application of effective microorganisms favored flower initiation in the test legume species that resulted in an increased number of pods, and hence the grain yield was increased. Xiaohou et al. (2001) reported increased yield and quality of various crops, fruits and vegetables in China due to foliar spray of effective microorganisms. A similar increase in yield of groundnut (*Arachis hypogaea* L.) due to foliar application of effective microorganisms was also reported by Yousaf et al. (2000) in Pakistan.

4. CONCLUSION

The results of the present study are in line with the findings of many earlier authors who reported that generally soil application of effective microorganisms has an insignificant or even a negative impact on crop growth and yield during the first year of application. The foliar application of these microorganisms enhanced nodulation and yield of the test plant in NPK fertilizers and green manure amendments. Based on the findings of the present study it is, therefore, suggested that these microorganisms should be applied as foliar spray in combination with appropriate soil amendments to enhance nodulation and yield of pea. Further studies regarding the effect of foliar spray on nodulation and yield of pea and other legumes should be carried out with different types of soil organic amendments as well as with lower doses of chemical fertilizers for sustainable agricultural development.

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